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Challenges and strategies in the reconstruction of frozen-hydrated yeast spores through X-ray diffraction microscopy

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Biological imaging is a demanding field that requires several important factors to be balanced in order to achieve the best results. High-resolution imaging is important to analyze the functionality of the sub-cellular structures, while the preservation of the sample in its natural state is crucial in order to avoid structural artifacts. This balance of high resolution and sample preservation is one of the key factors in achieving a faithful image of the sample with high precision.

Recently, X-ray diffraction microscopy (XDM) was applied to air-dried bacteria by Miao et al [1], and to freeze-dried yeast cells by Shapiro et al [2]. Both experiments showed the feasibility of XDM in biological imaging with a resolution of 30 nm. In XDM, the high penetration power of x-rays allows one to image a few-micron-size cell as a whole, eliminating the need to section thick samples. XDM also offers the potential of a lower radiation dose than lens-based methods. Together, these two benefits help to reduce the creation of structural artifacts compared to other imaging techniques. Yet to fully exploit XDM in biological imaging, the samples need to be imaged in the frozen-hydrated state (vitreous ice state). This cryo-preservation is advantageous because (1) samples are maintained in the hydrated state close to their natural state, (2) ice matrix reduces migration of radicals by locking the products of photo-absorption [3]. However, these advantages are tempered by the challenge of this type of sample preparation: achieving a vitreous ice state is not an easy task due to many factors, including a limited cooling rate upon rapid-freezing and ice contamination during the sample transfer. If the samples do not vitrify, crystalline ice will form, disrupt the sample, and contribute to the background scattering signal. This makes reconstruction more difficult, and results in reduced resolution. Progress in imaging frozen-hydrated biological samples will be presented through: (1) sample preparation toward vitrifying few-micron-thick yeast spores, (2) reconstruction of frozen-hydrated yeast spores, (3) challenges associated with the reconstruction and strategies to overcome these issues.

1. J. Miao et al, Proceedings of The National Academy of Science, 100(1):110–112, 2003.
2. D. Shapiro et al, Proceedings of The National Academy of Science, 102(43):15343–15346, 2005.
3. K.A. Taylor and R.M. Glaeser. Science, 186:1036–1037, 1974.